بسم الله الرحمن الرحيم

BIOCHEMISTRY

Lecture #29

Enzyme III Regulation

﴿ وَإِن تَتَوَلَّوْا۟ يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوٓا۟ أَمْثَٰلَكُم ﴾

اللهم استعملنا لنصرة دينك

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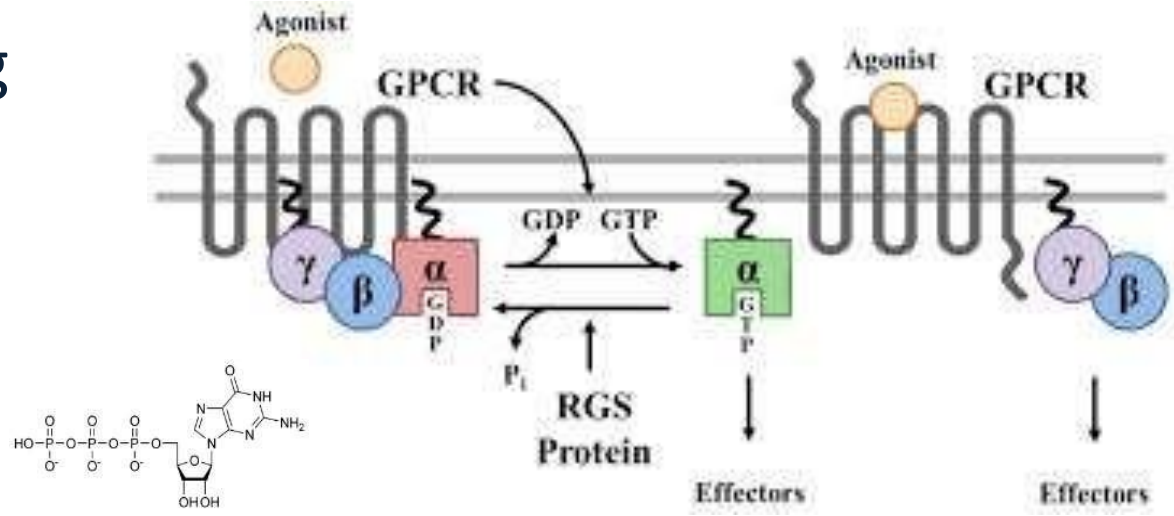
Large and small regulatory modulators

Trimeric large G proteins

Trimeric G proteins: a family of membrane-bound proteins causing changes inside the cell. They communicate signals from hormones, neurotransmitters, and other signaling factors through G protein-coupled receptors (GPCRs)

- **When they bind GTP, they are 'on', and, when they bind GDP, they are 'off'.**
- **The α subunit binds to effectors stimulating or inhibiting them.**

Alpha subunit:
Activated by → replacement of GDP by GTP (exchange).
Inactivated by→ hydrolysis of GTP to GDP.



G proteins

- They are called G proteins because they are regulated by GTP and GDP.
- **Two types of G protein:**

-Large trimeric G protein
-Small monomeric G protein

- G proteins are very important physiologically, the sense of smell, taste, vision and hearing ; all of them depend on G proteins.
- Also, almost **25%** of drugs in the market target G proteins.

- Trimeric G proteins are composed of a 3 molecules complex , alpha , beta and gamma . Alpha subunit is bound to a GDP molecule , and as long as it's bound to it ; it will also be bound to the (beta, gamma) complex.

- Trimeric (large) G proteins regulate the 7 transmembrane domains receptors . The binding of the ligand to the receptor causes the release of the alpha subunit because it binds to GTP rather than GDP (which is an exchange reaction).

- Notice that; the binding of (beta, gamma) complex to the alpha subunit is an **inhibitor** to G protein.
- Then, the alpha subunit binds to different enzymes and effectors ; activating them , and for that, alpha is considered as a **large regulatory molecule** (G protein is a regulator not an enzyme).
- Alpha subunit can bind to adenylyl cyclase that converts ATP→ cAMP, cAMP can then bind to protein kinase A.

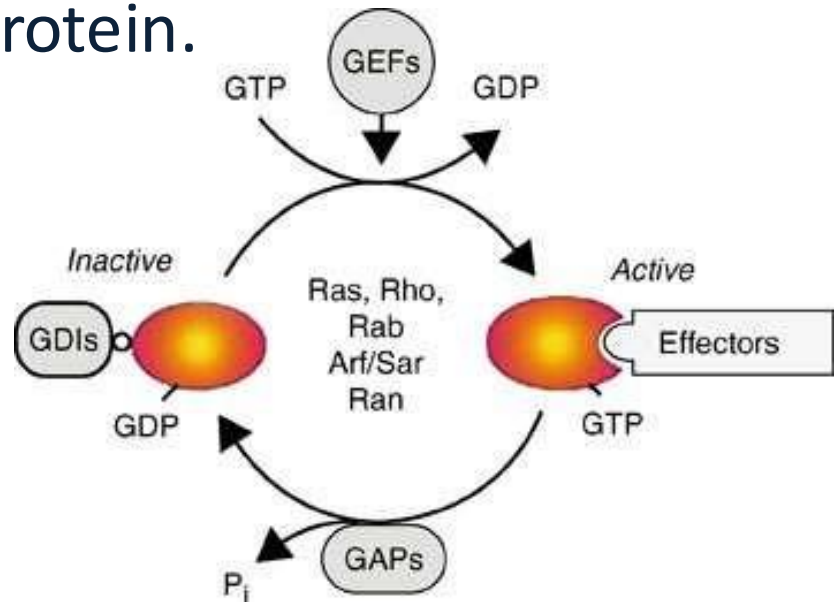
- Alpha subunit can be inactivated again by the **hydrolysis** of the GTP molecule into GDP and it rebinds to beta and gamma.

Large and small regulatory modulators

Small monomeric G proteins

- When GTP is bound, the conformation of the G protein allows it to bind target proteins, which are then activated or inhibited.
- The G protein hydrolyzes a phosphate from GTP to form GDP, which changes the G protein conformation and causes it to dissociate from the target protein.
- GDP is exchanged for GTP, which reactivates the G protein.

They bind to enzymes, like **kinases** that phosphorylate other enzymes, those other enzymes will also phosphorylate other enzymes in a **signal transduction** process.



Irreversible covalent modification
Proteolytic activation

Zymogens

They are proenzymes that require proteolysis (lysis/cleavage of specific regions of their protein structure) in order to be active.

- Zymogens or proenzymes are inactive precursors of enzymes.
- Activation is done by irreversibly removing part of the enzyme (usually known as the pro region present at the N-terminus).

The term (pro-) before the enzyme's name means that this enzyme is inactive.

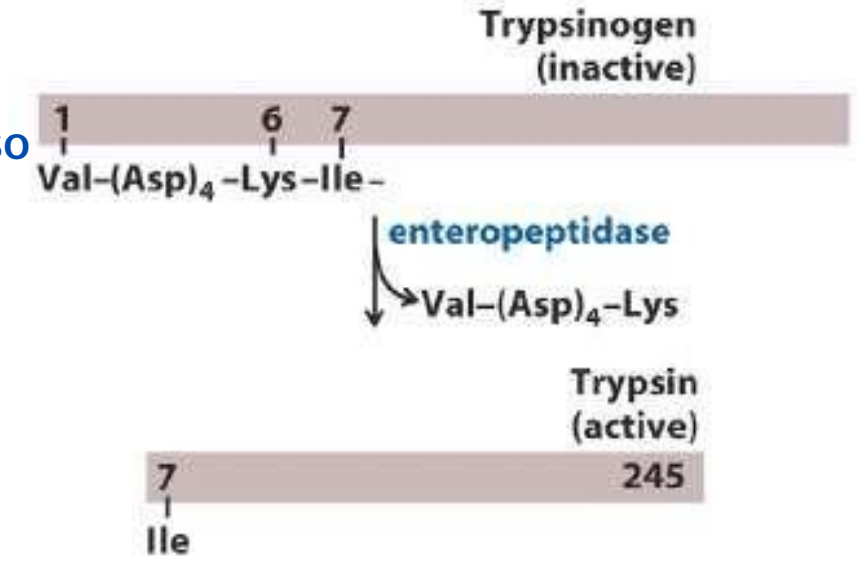
- Examples: digestive enzymes such as chymotrypsin, trypsin, and pepsin that get activated when food is ingested.

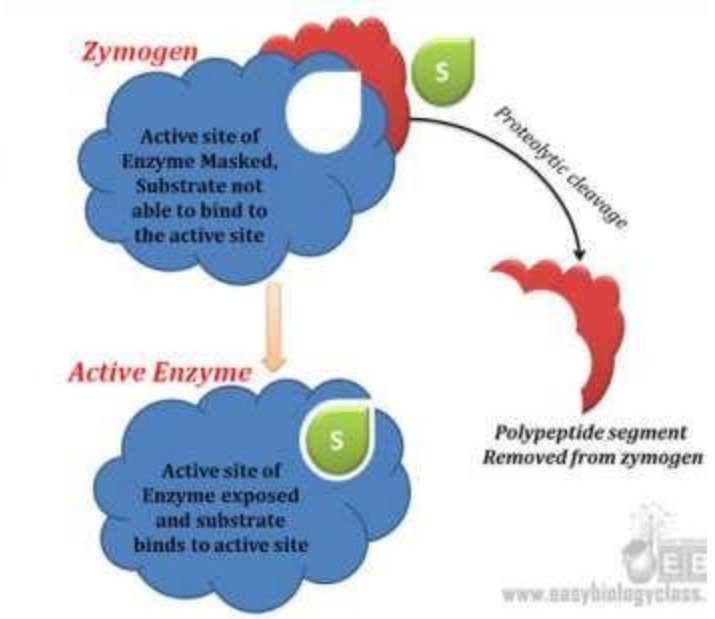
Lysosomal (digestive) enzymes are zymogens.

  - Trypsinogen (zymogen) is activated via removal of the first six amino acids at the N-terminus.

Zymogens are present in the pancreas waiting for a signal, so they can be released to the intestine where they get activated.

This signal comes immediately when you eat :)





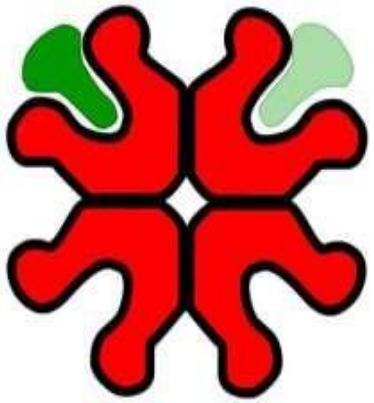
Regulation: conformational changes

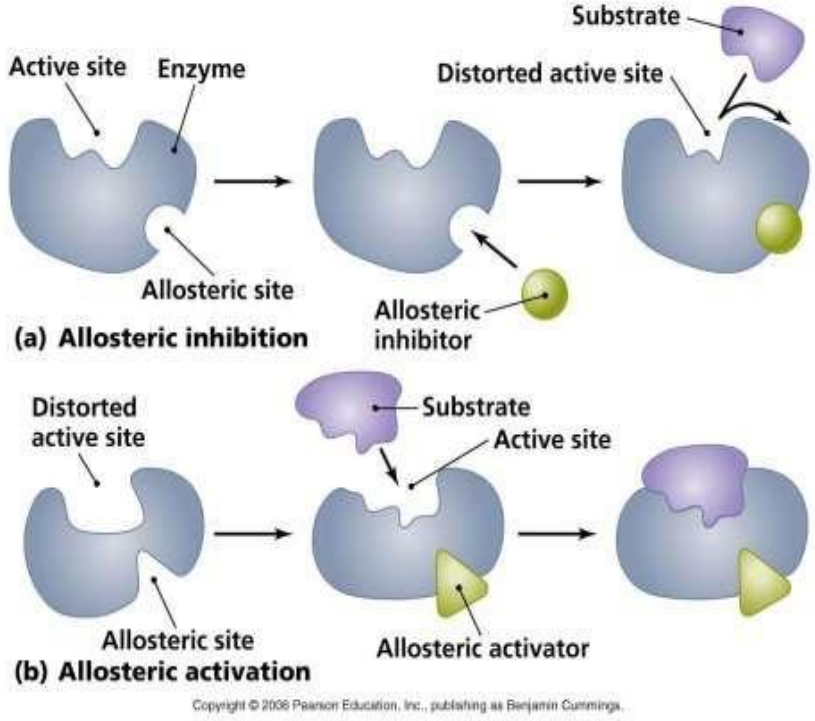
- These regulatory mechanisms include
  - Allostery
  - Covalent modulation
  - Protein-protein interactions between regulatory & catalytic subunits or between two proteins;
  - Proteolytic cleavage
- Rapidly change from inactive to fully active enzyme.

Allosteric regulation

Allosteric enzymes and their modifiers

- Allosteric enzymes are multi-subunit proteins; Quaternary structure
  - One subunit contains the active site (catalytic subunit) and another containing the regulatory site (regulatory subunit).
  - Multiple active sites can exist on multiple subunits.
- The binding of regulatory molecules triggers conformational changes in the active site via modifying non-covalent interactions. Like in hemoglobin when it binds to oxygen.
- Allosteric enzymes bind modifiers at the allosteric site, a site that is physically separate from the catalytic site.
  - A negative allosteric modifier (inhibitor) causes the enzyme to have less activity. The substrate will not fit.
  - A positive allosteric modifier (activator) causes the enzyme to be more active. The substrate fits perfectly.

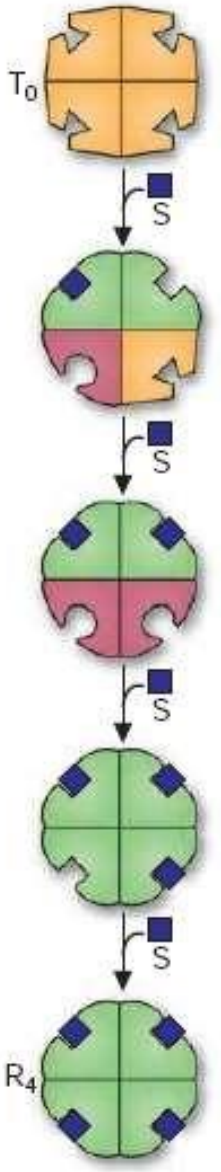




More on modifiers

- When the modifier is a molecule other than the substrate, then it is known as heterotropic.
- If the modifier is same as the substrate, then it called homotropic.
  - The binding of the substrate causes the enzyme to become more active and binds to a second substrate at a different active site with more ease.
  - This is called "positive cooperativity". T to R conformation
  - There is also negative cooperativity.

Positive cooperativity means that the binding of one substrate to the active site of one subunit, makes it easier for another substrate to bind to the active site of another subunit.



Remember! When a negative regulator binds to the enzyme , it doesn't become fully inhibited, just the activity of it becomes low.
Can oxygen binds to a T-hemoglobin? Yes, but it binds with low affinity.
Can an allosteric enzyme be active in the T-state? Yes, but it's not really that active.

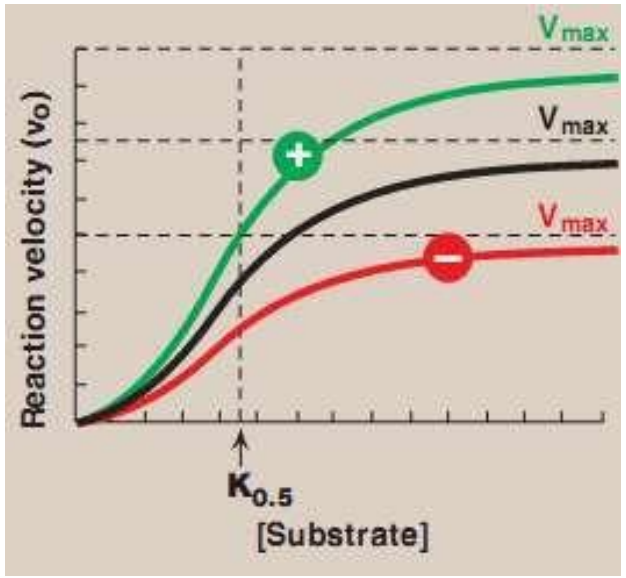
Types of allosteric enzymes

- The Michaelis-Menten model cannot explain the kinetic properties of allosteric enzymes.
- $K_{0.5}$ is used instead of K_M.

It indicates the affinity of the enzyme.
It's the concentration of the **substrate** when the velocity of the reaction equals **half** the Vmax.

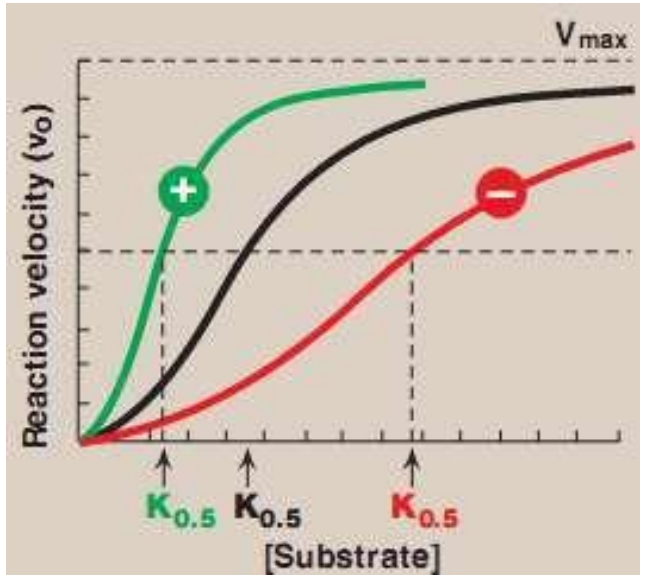
Allosteric enzymes have a **sigmoidal** plot the same as hemoglobin , unlike Michaelis-Menten's hyperbolic plot.

V system



Same K0.5, Different Vmax.

K system



Different K0.5, same Vmax.

The binding of the allosteric effector can change :**either** Vmax (V system) **or** the affinity (K system).
(The Professor said he doesn't know any effector that changes them both at the same time).

Note near-hyperbolic plot with activators

Allosteric enzymes and metabolism

Recall that: the regulation of the enzyme is **gradient** , it changes it's shape gradually, not in an on/off situation.

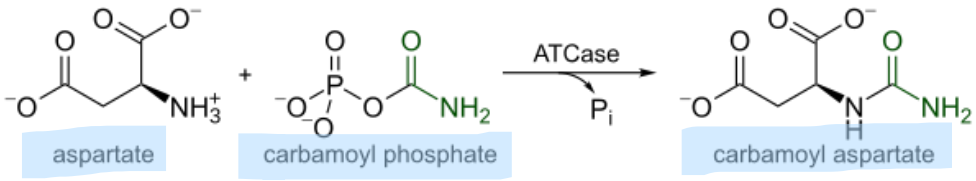
- Allosteric inhibitors usually have a much stronger effect on enzyme velocity than competitive and noncompetitive inhibitors.
- Allosteric enzymes are not limited to regulation through inhibition whereby allosteric effectors may function as activators.
- The allosteric effector needs not bear any resemblance to substrate or product of the enzyme.
- The effect of an allosteric effector is rapid occurring as soon as its concentration changes in the cell. It's also a **huge** effect on the enzyme's activity.
  - Feedback regulation of metabolic pathways by end products or by signal molecules that coordinate multiple pathways.

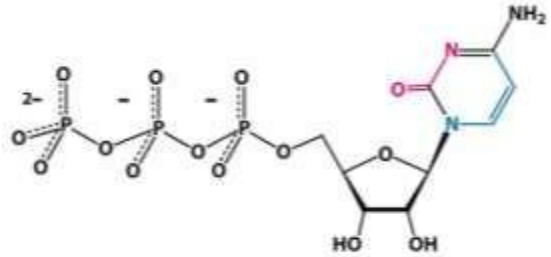
Which is a difference between them and Michaelis-Menten's regulators that only function as inhibitors.

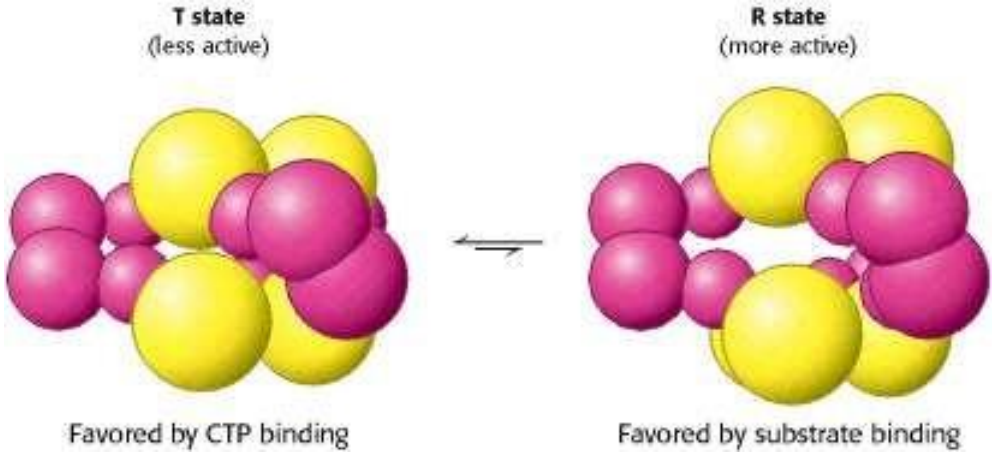
Aspartate transcarbamoylase

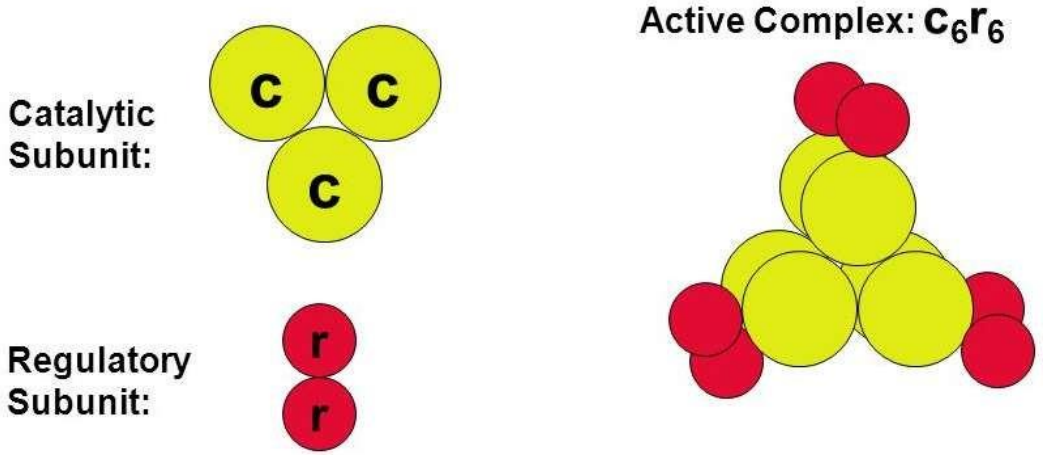
It's responsible for the first step of the formation of CTP (which is a pyrimidine).

- Aspartate transcarbamoylase (ATCase) catalyzes the first step in the synthesis of pyrimidine nucleotides.
- ATCase consists of 12 polypeptide chains: six catalytic subunits (two trimers) and six regulatory subunits (three dimers).
- It exists in two forms: T state (less active) and R state (more active).









Aspartate transcarbamoylase-regulation

regulated by

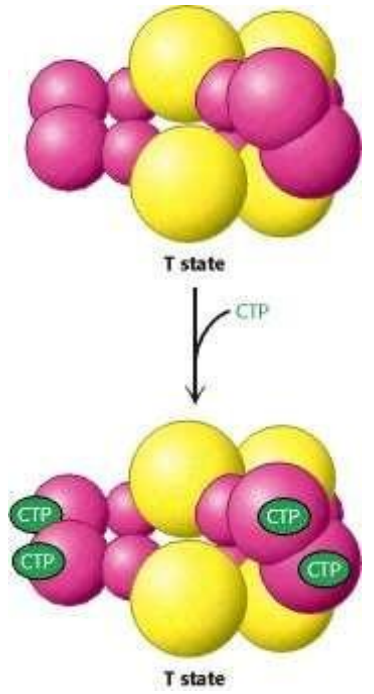
- ATCase is inhibited by CTP, the end-product

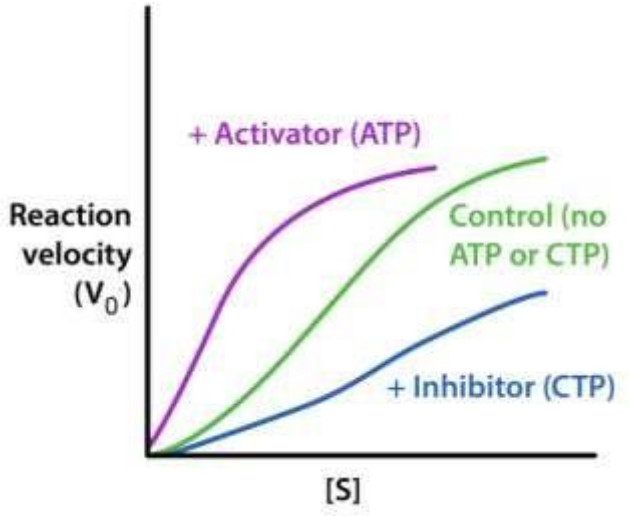
Binds to the enzyme and makes it less active
(tells the enzyme there's enough of CTP)

  - inducing a major rearrangement of subunit positions
  - stabilizing the T state of the enzyme.
  - decreasing binding affinity for Asp (substrate) at active sites on catalytic subunits
  - increasing K0.5 (K system)

Vmax & Kcat aren't affected

    - Note: a non-competitive inhibitor changes K0.5
    - On the other hand, ATP, a purine, heterotypically activates the enzyme in order to balance the rate of synthesis of purines and pyrimidines in cells.





- Allosteric activator >> ATP (purine) >> because the cell needs balance between purines and pyrimidines >> so if CTP (pyrimidine) was high, CTP itself will inhibit (lower the activity of) the enzyme to have less pyrimidines >> but if ATP was high, it will activate the production of CTP to have more pyrimidines as purines

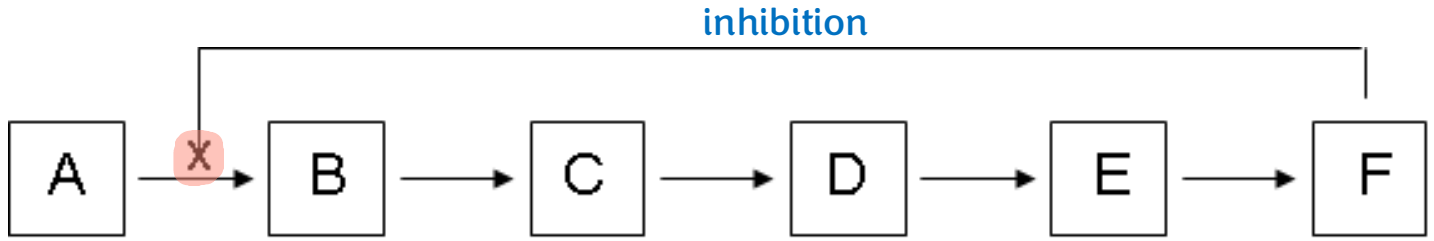
Modes of metabolic regulation

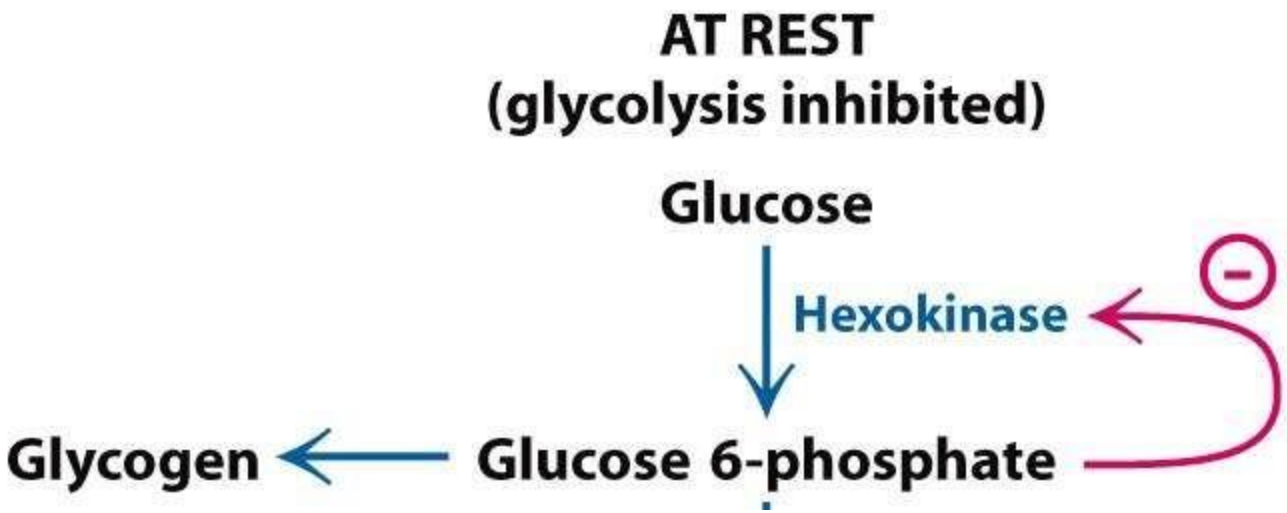
" لا إله إلا أنت سبحانك إني كنت من الظالمين"

Feedback inhibition

- Feedback inhibition or **negative feedback regulation**: an enzyme present early in a biochemical pathway is inhibited by a **late product** of pathway.

1- An end product will affect an enzyme in the beginning, it will stop/inhibit the enzyme because the cell has enough of the product.





2- direct product of the reaction inhibits the enzyme that makes it

AKA, product inhibition

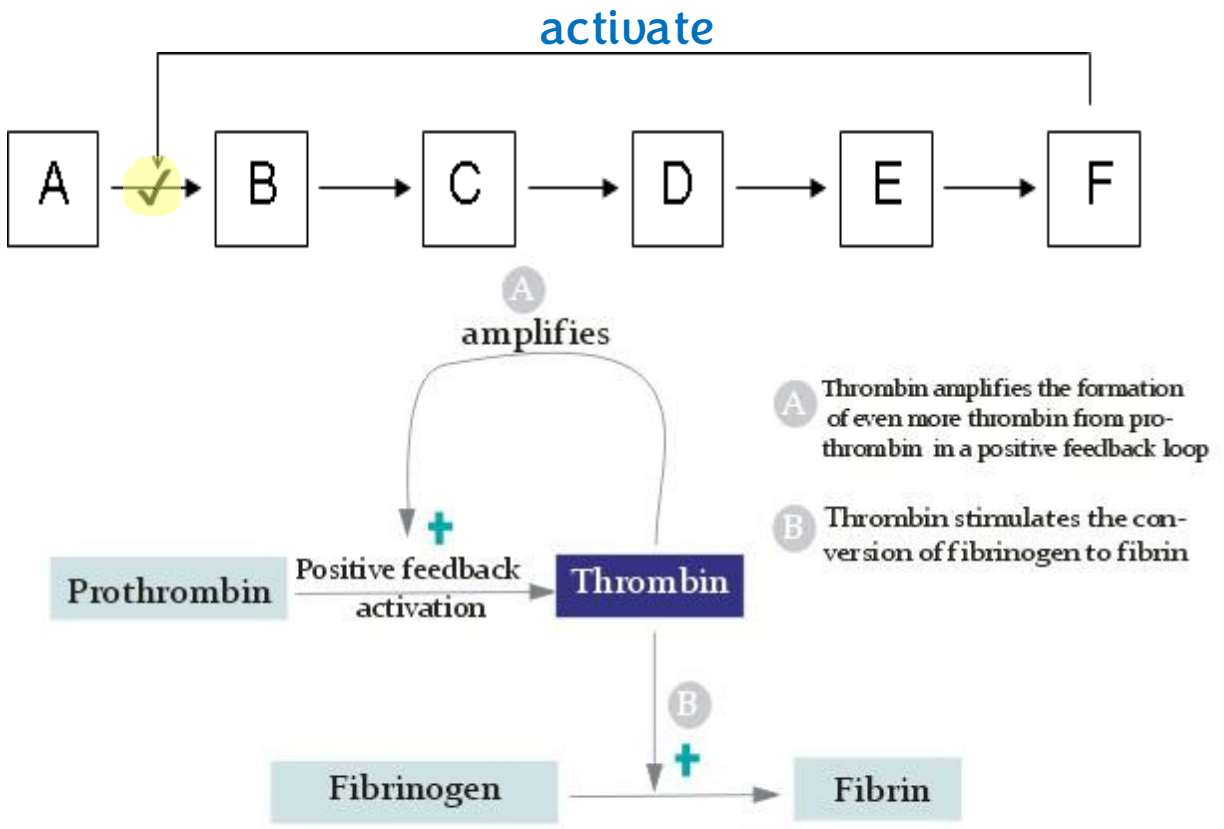
Negative feedback

So, there are two types of feedback inhibition:
- End-product inhibition
- Direct product inhibition

Feedback activation

- Positive feedback regulation: a product stimulates the activity of an enzyme.

Example:
blood coagulation, there's bleeding that needs to be stopped immediately, so when a **late product** activates an **early enzyme**, the coagulation will be **accelerated**, to seal the injury and stop the bleeding.



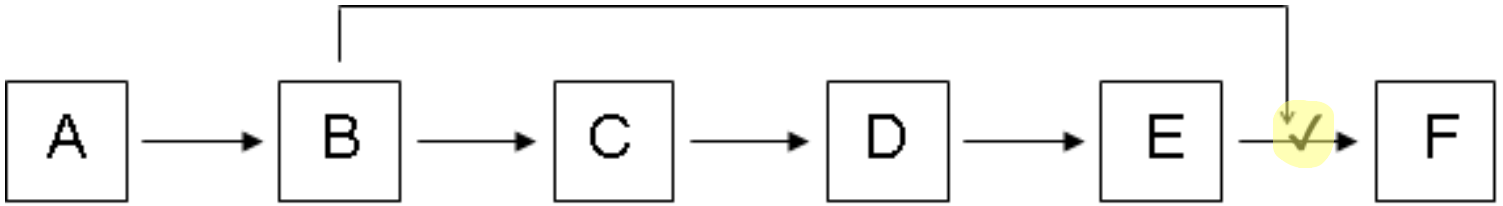
Positive feedback activation of more prothrombin into thrombin

Feed-forward activation

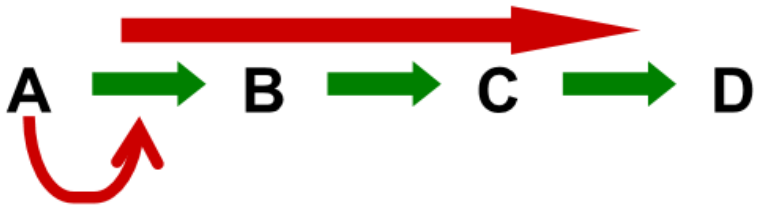
Why? To accelerate the reaction

E.g.1: glycolytic pathway, converting Glucose into Fructose 1,6 bisphosphate which will activate that last enzyme in the reaction chain.

- Feed-forward regulation: a **substrate** produced early in a pathway **activates** an enzyme downstream of the same pathway.



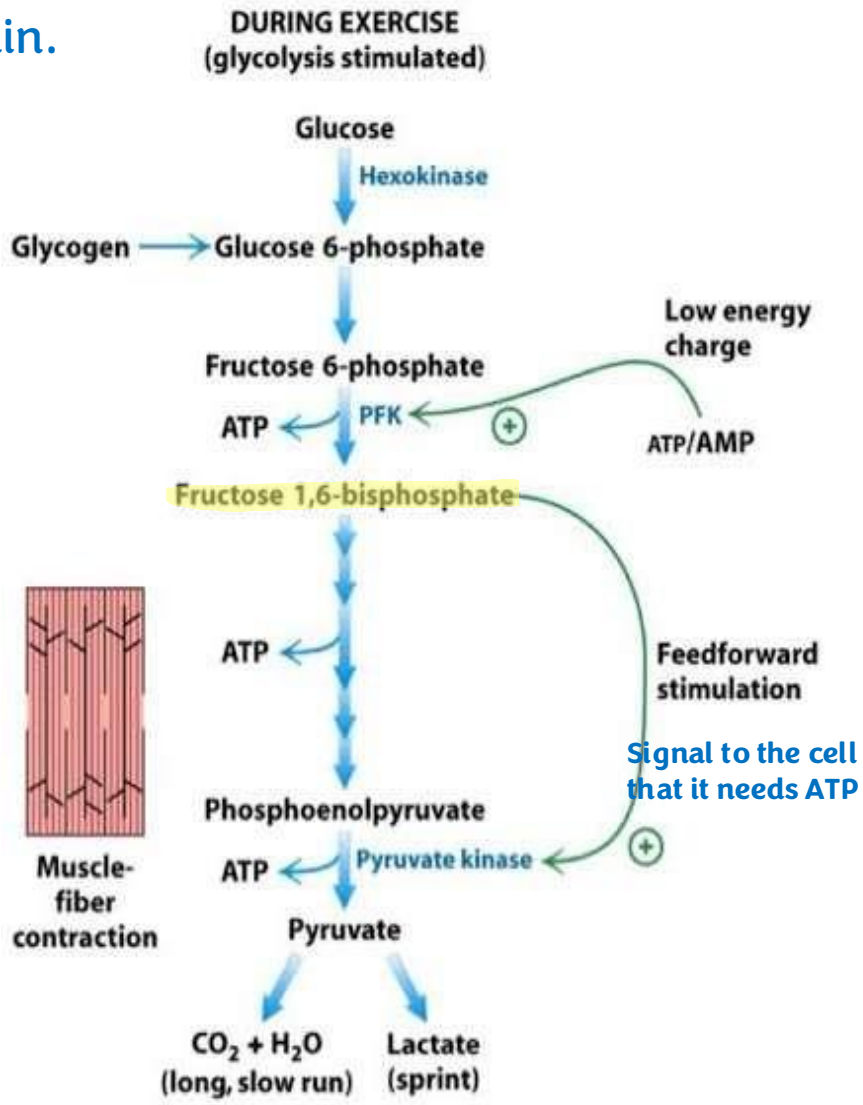
- Glycolysis
- Poisoning



"Oh no! Get this poison out of here!"

"Alot more substra coming through!"

E.g.2: to get rid of a poison (we need the rxn to be fast) so, sensing A (the poison) will accelerate the rest of the reactions.



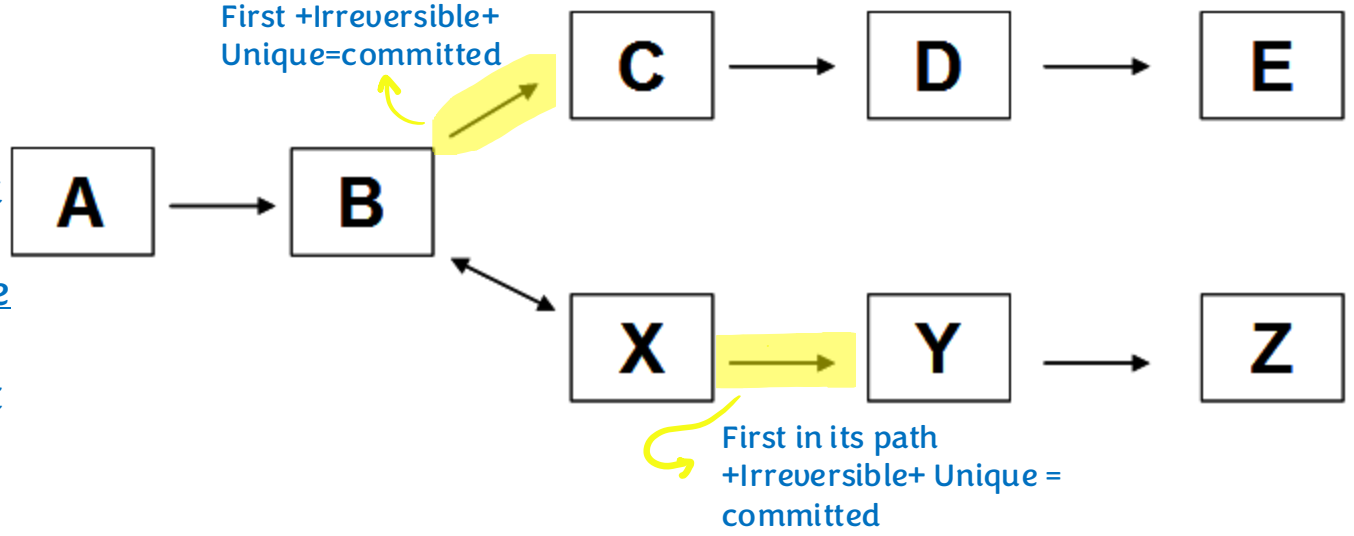
A committed step

Needed for the metabolism course!

- A committed step in a metabolic pathway is the **first irreversible** reaction that is **unique** to a pathway and that, once occurs, leads to the formation of the final substrate with no point of return.
- Committed steps are **exergonic reaction**.
- For example, the committed step for making product E is (B → C), not (A → B).

A step that once it happens, the reaction has to move a certain pathway & commit to it,
A committed step is (ALL not either):

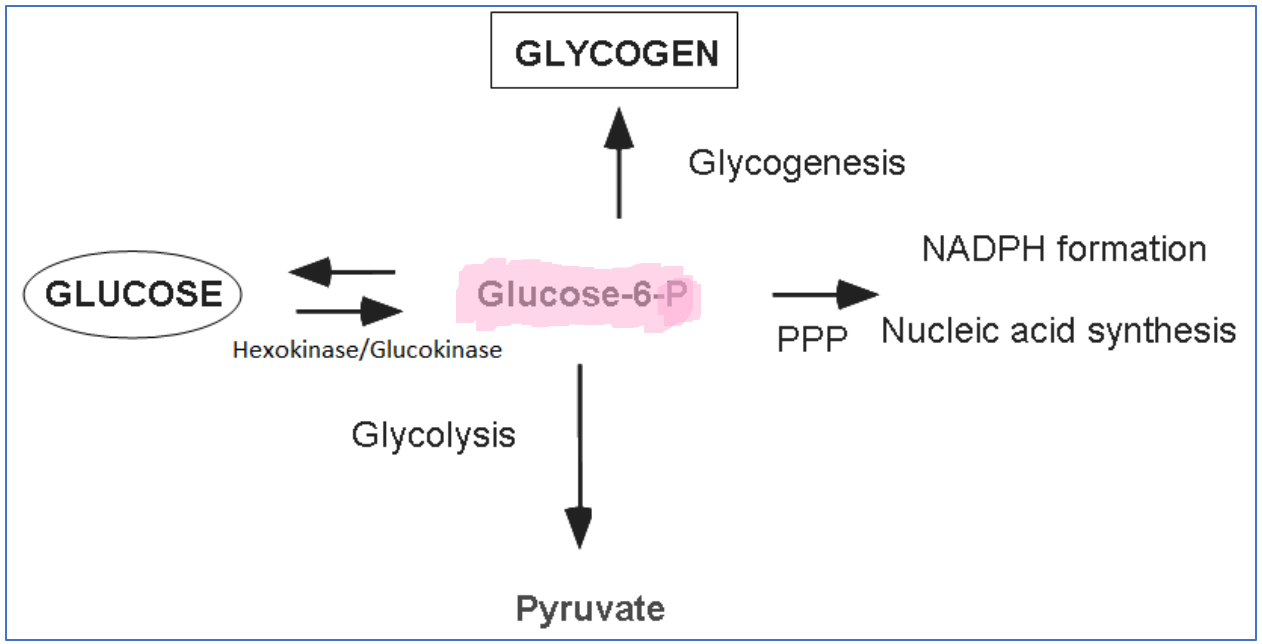
1. Is the first step in the pathway that satisfies both (2&3)
2. Can't return to the substrates- irreversible (Here, B\X rxn is not a committed step because you can go back to B)
3. Can't produce different products-Unique (Here, in A\B, there are 2 alternate paths\products that could occur> it's not committed)

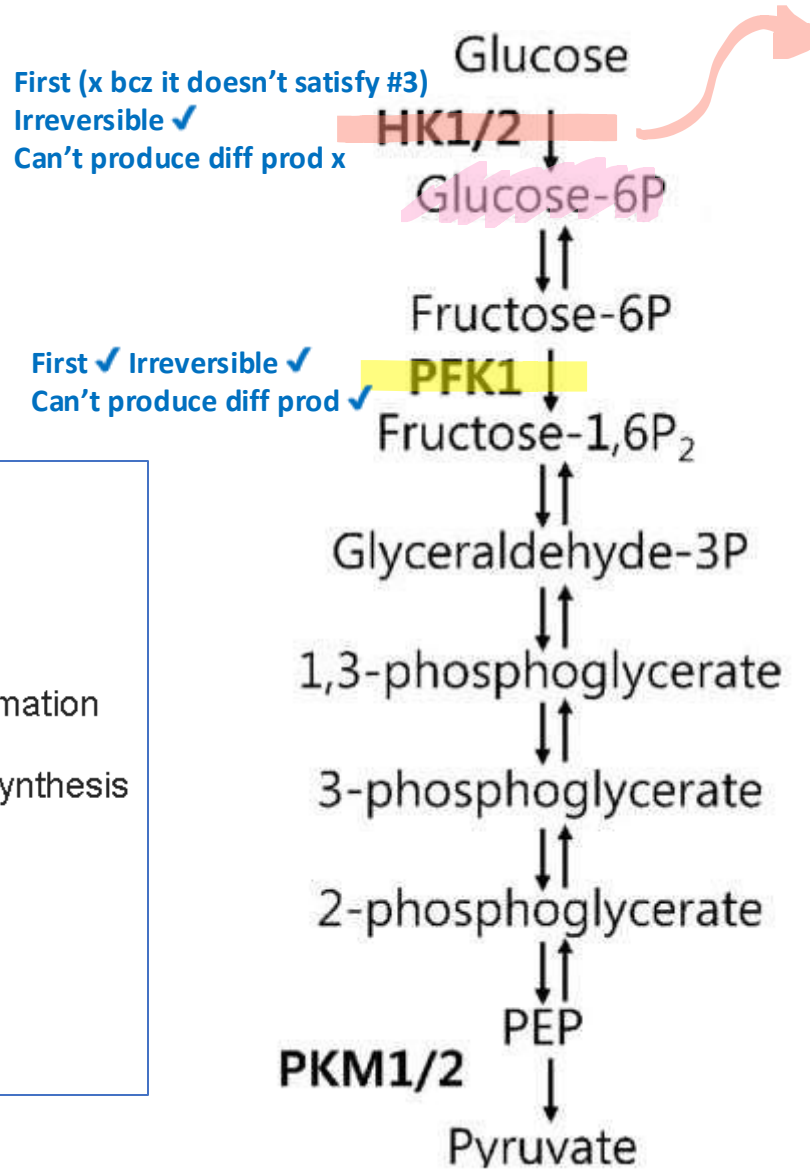


"اللهم أنت عضدي ونصيري ، بك أحاول وبك أصاول ، وبك أقاتل"

PFK, not HK/GK, is the committed step

The step that is catalyzed by phosphofructokinase is the committed step, if this reaction happens, the pathway won't go backwards, it will continue (no matter if the reactions after are reversible) until it gets to pyruvate.





Is the rxn that is catalyzed by hexokinase the committed step in converting glucose to pyruvate? NO

Why? Glucose-6P has options, could become pyruvate, could go back to being glucose, glycogen, ribose. So phosphorylation does not equate to a committed step in this pathway. (look at the pic on the far left).

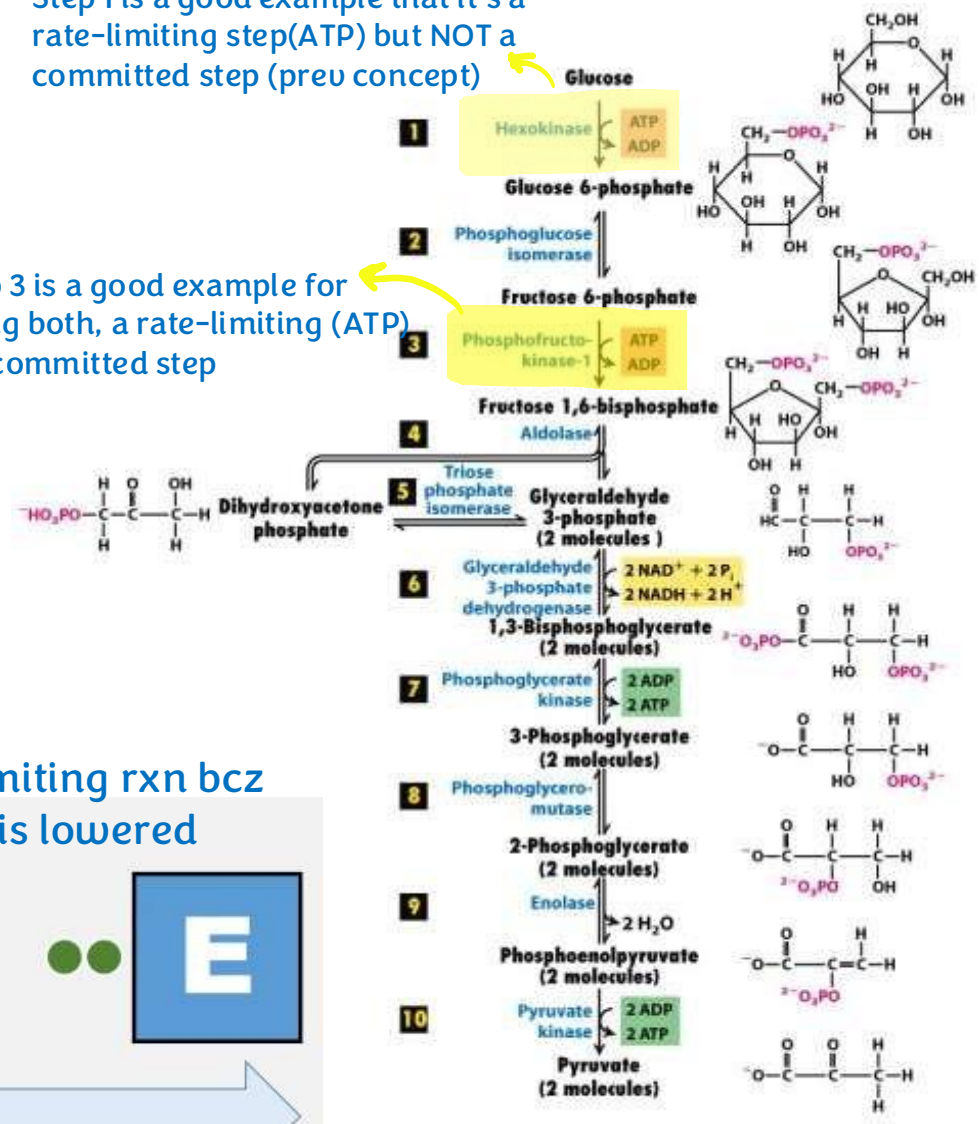
Rate-limiting reactions

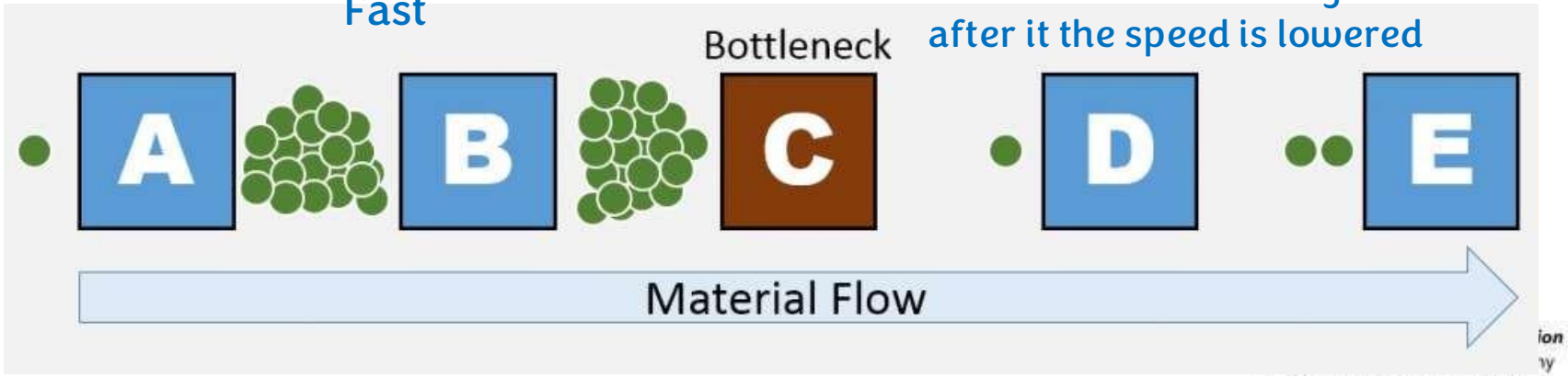
Needed for the metabolism course!
A step that limits/lowers the rate/speed of the reaction chain

- Rate-limiting reactions slow down rate of reactions because:
  - **requirement for high amount of energy**
    Any rxn that requires ATP is slow
  - strict regulation of enzymes
  - **high Km** values of enzyme towards its substrate
    High Km = Low affinity= slow rxn
- These reactions are also **usually**, **but not necessarily, committed steps.**

Step 1 is a good example that it's a rate-limiting step(ATP) but NOT a committed step (prev concept)

Step 3 is a good example for being both, a rate-limiting (ATP) & a committed step





C>D is the rate-limiting rxn bcz after it the speed is lowered

Enzymes in disease diagnoses

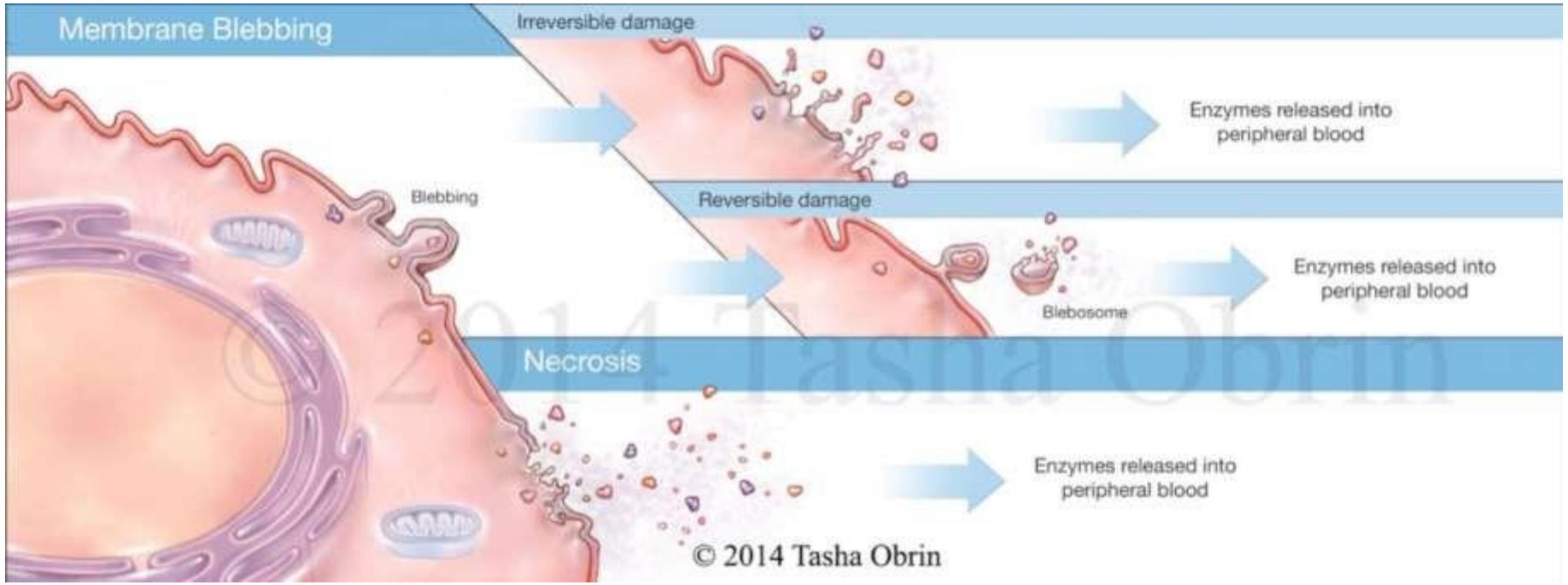
Concept

Cells die>> release their content(everything inside) >> normal >> its normal to find liver enzyme in the blood for example (bcz cells die at a moderate rate)

Cell rupture/injury>> the level of cell enzyme will increase in the blood >> in this case the level of liver enzymes will increase in blood>> we can tell there's a liver injury/infection/etc

This also applicable on brain tissue, kidney, etc

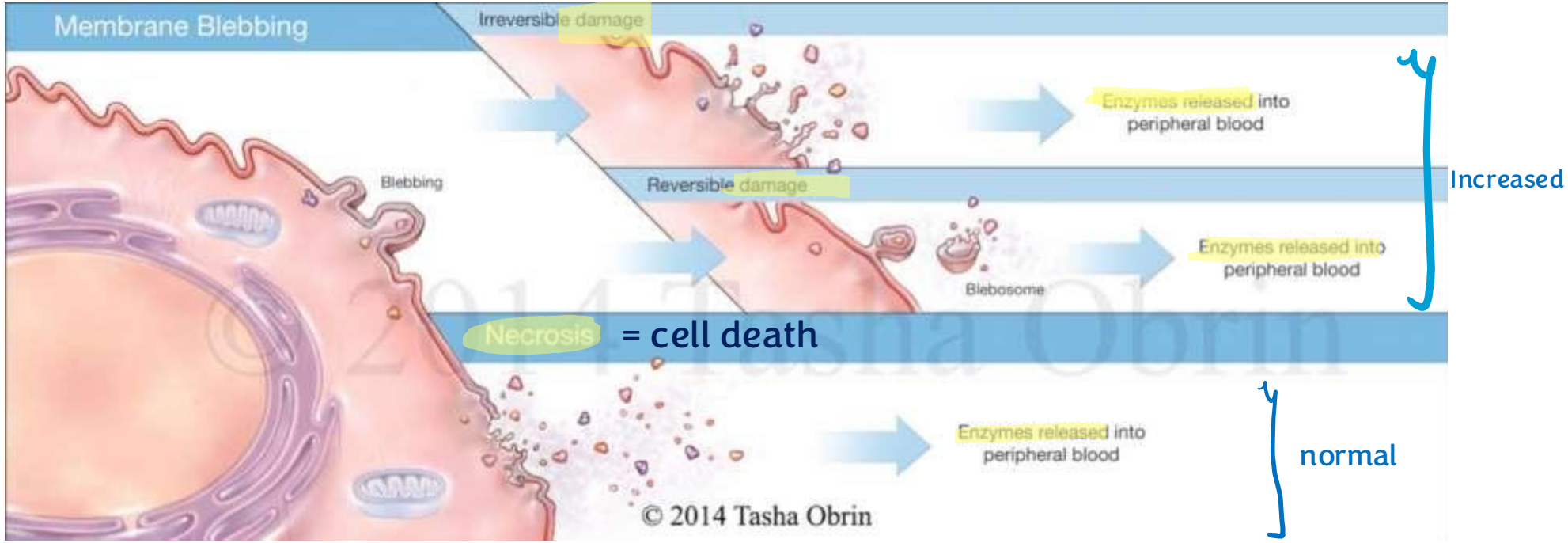
- The presence of enzymes in serum indicates that tissue or cellular damage.
- The measurement enzyme amount in serum is of **diagnostic significance.**



Another example:
Cancer cell's increased proliferation will increase the level of its markers in the blood

Concept

- There are many markers for Heart Attacks, what happens during that?
- Clogging/plug of the flow of the blood >> hypoxia >> cells die and rupture >> release of heart muscle's enzymes
- Normally, these proteins aren't supposed to come out of the heart, but it just happens that there's an injury
- So, if these markers are found in blood>> probability of Heart attack



Example: Creatine phosphokinase (CPK)

Example of enzymes functioning as markers for diseases.

- CPK is found primarily in heart and skeletal muscle as well as the brain.
- **Three tissue-specific isozymes** of CPK:
  - CPK3 (CPK-MM) (mainly in muscles)
  - CPK2 (CPK-MB)
  - CPK1 (CPK-BB) (mainly in brain)

Regarding the prev silde>> there are many proteins/enzymes that are present both in the heart muscle and skeletal muscles, right? NO
These are isozymes that differ from brain-muscles-heart etc
One of these enzymes is CPK.

Serum	Skeletal Muscle	Cardiac Muscle	Brain
0 trace BB <6% MB >94% MM	0 trace BB 1% MB 99% MM	0% BB **20% MB** **80% MM**	97% BB 3% MB 0%MM

Based on this table, there's a significant amount of MB in cardiac tissue >> if there happens to be an injury in the cardiac muscles, & enzymes were released into the blood >. The enzymes that will increase are both MM (more) MB (less but significant) >> so to look for heart attacks we need to look at the levels of CPK-MB>> as the only way it would increase significantly is through cardiac muscle rupture (if only MM increased>> it's a skeletal muscle issue)

There's also troponin, but it's a protein not an enzyme

For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V1 → V2			
V2 → V3			

Additional Resources Used:

1. Mark’s Biochemistry pgs(344)(358-366)
2. Youtube Video #1
3. Anything else…

رسالة من الفريق العلمي:

اللهم افتح لي أبواب حكمتك، وانشر عليّ رحمتك، وامنن عليّ بالحفظ والفهم والتوفيق، سبحانك لا علم لنا إلّا ما علمتنا، إنّك أنت العليم الحكيم.

"اللهُم ارحم أمواتنا وأموات المسلمين و اجعل أرواحهم من الأرواح المُنعمة المُبشرة بالخلود في جنة الفردوس يارب العالمين".